No. 5.

(*NEW SERIES.*)

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

SPECIFICITY OF ANTIVENOMOUS SERA,

BY

GEORGE LAMB, M.D., (GLASG.) CAPTAIN,
(*Indian Medical Service*).

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SPECIFICITY OF ANTIVENOMOUS SERA.

THE question of the specificity of antivenomous sera is a question of great importance and interest both from the practical and from the theoretical standpoint. Let us first consider the practical aspect of the question.

It is at once evident that, should it be definitely proved that the serum of an animal immunised with the venom of one species of snake was not antitoxic to the poison of any species, except of that species the venom of which had been used for the purpose of immunisation, the difficulties of the therapeutics of cases of snake-bite would be enormously increased. Even if it were possible to prepare sera specifically antitoxic to the venoms of all the species of poisonous snakes, a moment's consideration will show that the difficulties would not be overcome by supplying to the public a different serum for each variety of snake venom. Thus, in India[1] alone there are four genera of terrestrial snakes, which can be said to be dangerous to man, *viz.*, Naia, Bungarus, Vipera, and Echis. Further, of some of these genera there are several species. The genus Naia contains two Indian species, the genus Bungarus five species, and the genus Vipera two species. The genus Echis has only one species which occurs in India, *viz.*, Echis carinata. Some of these species, however, are rare and therefore do not threaten much danger to man. Nevertheless, at the lowest estimation we should have to supply six different antivenomous sera to meet the wants of India alone, *viz.*, sera for the venoms of Naia tripudians (cobra), Naia bungarus (king cobra), Bungarus fasciatus (banded krait), Bungarus cæruleus (common krait), Daboia Russellii (chain viper), and Echis carinata (phoorsa).

Further observations, however, might possibly show that the serum prepared with the venom of one species of snake was antitoxic for the venom of another species of the same genus. If such an hypothesis were found to be true, we should still have to prepare four antitoxic sera for the venoms of the four most poisonous genera of Indian snakes, granting always, of course, that the serum prepared with the venom of one genus was not efficacious against the poison of any other genus.

Now, when a person, especially a native of India, is bitten by a snake, he is rarely able to tell the species of the snake which has inflicted the bite. And, further, to be of much practical utility an antivenomous serum must be injected before any symptoms of poisoning have set in. The medical man, therefore, who is called on to treat a case of snake-bite with antitoxin is not, as a rule, in a position to form an opinion, either from the history of the case or

from the symptoms as to the nature of the venom which has been injected. He would have, then, either to use one of the antitoxic sera at hazard or to inject the whole of them at once. For obvious reasons it is certain that neither of these methods would recommend itself either to the patient or to the medical man as a sound and reliable therapeutical measure.

The difficulty might be got over in one of two ways. It might be got over either by immunising animals against the venom of each species of snake separately and then mixing the various sera procured in this way, or by immunising, if this were possible, animals with a mixture made up of definite proportions of each venom. It is, however, premature to speculate further in this direction, before we have shown that it is necessary to overcome the difficulty, and before many preliminary experiments have been made. Nevertheless, the considerations put forward above show the great practical importance of the question under discussion.

The theoretical aspect of the problem is of no less importance and interest than its practical aspect. Calmette[1] has all along held that all snake venoms are alike in physiological action, and that, in consequence, the serum which he sends out from Lille, prepared as it is chiefly with cobra venom, is active against the venoms of all species of snakes. These sweeping statements of Calmette have not stood the light of further and more recent research. They have now been shown to require considerable modification. In the first place, I have shown in a series[2] of papers that the venom of the cobra differs from that of the daboia in every detail of physiological action. In the second place, in Australia it was first shown by Martin[4] of Melbourne that Calmette's serum was unable to preserve animals against injections of the poison of the tiger snake (Hoplocephalus curtus). Subsequent experiments[5] in Sydney have demonstrated a like fact as far as the poisons of other Australian snakes are concerned. In India, also, along with Dr. Hanna[5] I have conclusively shown that, while Calmette's serum is active for cobra venom, it has no neutralising effect for the venom of Daboia Russellii. When we consider, as I have stated above, that the venom of the cobra has in every respect a different physiological action from the poison of the daboia, such a result was only to be expected. It is evident, therefore, that if it were definitely shown that antivenomous sera were specific, Calmette's assertions would be conclusively disproved.

The question under discussion is further of theoretical interest in view of Martin's hypothesis,[7] that all venoms contain two or more poisonous proteids, and that in the varying proportions of these in venoms from different species of snakes may be found the explanation of the different effects which follow the inoculation of the serveral poisons. It is unnecessary here to enter into the facts I have elsewhere[8] put forward which go to disprove this theory. It is sufficient to point out that, should it be shown that the serum of an animal immunised with the venom of one species of snake was quite inactive against the venoms

of other species, this hypothesis of Martin would receive its death blow. We shall return to this question later on.

With this introduction we may now pass on to examine the data which bear on the question under consideration.

In a recent paper[9] on the serum therapy of snake bite Dr. Tidswell of Sydney has correctly pointed out that Calmette's serum, inasmuch as it is prepared with a mixture of snake venoms, in which mixture, however, cobra venom greatly preponderates, is not strictly adapted for the settlement of specificity, and that it was necessary for this purpose to possess a serum prepared with one single kind of venom and to test its efficacy against the same and other kinds of venom. In this same paper he gives some most interesting results which he obtained working with the serum of a horse immunised with the pure venom of the Australian tiger snake (Hoplocephalus curtus). He showed that 0·4 c.c. of this serum was sufficient to neutralise ten lethal doses, *viz.*, 0·00059 grammes, for the rabbit of the particular venom used to immunise the horse. He further showed that this same serum failed completely to neutralise the poisons of the brown and black snakes and also that of the death adder. The outcome, in short, of Tidswell's observations "is such as to indicate that the serum is specific in its action, operative only against the venom by means of which it is prepared."

The observations which I have now to put forward further extend my original observations, mentioned above, made with Calmette's serum and cobra and daboia venoms. They, also, further extend the observations made by Tidswell with the serum prepared with the poison of Hoplocephalus curtus. It is unnecessary here to reprint the details[10] of the experiments made with Calmette's serum and cobra and daboia poisons. It will be sufficient to recall the main facts demonstrated by these experiments, *viz.*, that the serum sent out from the laboratory at Lille was able to neutralise, to a certain extent, the venom of the cobra, but was quite inactive against the venom of Daboia Russellii.

The direction in which these observations were now extended was to test Calmette's serum with the venoms of two other species of Indian snakes, *viz.*, Bungarus fasciatus and Echis carinata. Bungarus fasciatus is a snake belonging to the genus Bungarus, closely allied to the genus Naia, of which the cobra is a species. It is interesting in this connection to note that the genus Naia and the genus Bungarus belong to the same sub-family of Colubridae, *viz.*, Elapinæ, in which sub-family, also, naturalists classify the bulk of the ophidian fauna of Australia. Further, all the members of the sub-family Elapinæ are poisonous. Echis carinata belongs to the genus Echis, a genus closely allied to the genus Vipera, of which genus Daboia Russellii is a species. The genus Echis and the genus Vipera belong to the same sub-family of Viperidæ, *viz.*, Viperinæ.

Let us first consider the experiments made with Calmette's serum and the venom of Bungarus fasciatus. As a preliminary measure it was necessary to

determine the minimum lethal dose of this poison when injected intravenously into rabbits. A reference to series No. I of the protocols will show that 0·7 milligramme per kilo. was the smallest amount which could be depended upon to cause the death of the animal. Two series of observations were then made with Calmette's serum and this venom.

In the first series, *vide* protocols, series No. II A, four milligrammes per kilo., *viz.*, about six lethal doses, were used as the test dose. This test dose was mixed *in vitro* with different quantities of serum. The mixture was allowed to stand at laboratory temperature (about 25°C.) for half an hour. It was then injected into the marginal vein of the rabbit's ear. A glance at the table appended will show that even 5 c. c. of serum were of no avail to save the life of the animal, the rabbit which received this amount of serum along with the test dose dying after practically the same interval of time as the control animal.

In the second series of experiments made with Calmette's serum and the venom of Bungarus fasciatus, *vide* protocols, series No. II B, two milligrammes of venom per kilo., *viz.*, about three lethal doses, were used as the test dose. With this exception the same technique was employed as in the previous series of experiments. It will be seen from the protocols that even with this small multiple of the lethal dose, 5 c. c. of serum were not able to preserve the life of the animal. The rabbits, however, which received 4 c. c. and 5 c. c. of serum lived somewhat longer than the control animal. When we consider that the test dose in these cases was such a small multiple of the minimum lethal dose, this result does not appear to me to influence the conclusion to which we are led by these two series of observations, *viz.*, that Calmette's serum has practically no neutralising power for the venom of Bungarus fasciatus.

I have next to put on record the observations which were made to ascertain if the Lille serum could neutralise the venom of Echis carinata. In this case, also, it was necessary as a preliminary measure to determine the minimum lethal dose of this poison for a rabbit when injected intravenously. Injected in this way the venom of Echis carinata causes death in the same manner as the poison of Daboia Russellii, that is to say, by widespread intravascular thrombosis." The smallest quantity which could bring about this fatal thrombosis was found to be 0·05 milligramme per kilo. A reference to the protocols (series No. III) will show that one rabbit which received this amount died in two and a half minutes, and another rabbit, which received a like quantity, lived for eleven and a half minutes. Rabbits which received less than 0·05 milligramme per kilo. recovered.

In order to give the serum every advantage the test dose of venom, which was used to test Calmette's serum, was only a single lethal dose, *viz.*, 0·05 milligramme per kilo. Two experiments, *vide* protocols, series No. IV, were performed. In the first instance the test dose of venom and 4 c. c. of serum were mixed *in vitro*. This mixture, soon after its preparation, was injected

into the marginal vein of the ear of a rabbit. The rabbit died in two minutes. In the second experiment 5 c. c. of serum were injected into the marginal vein of one ear. A few minutes afterwards the test dose of venom, *viz.*, a single lethal dose, was injected into the marginal vein of the other ear. This animal likewise died in about two minutes. From these two observations we can at once conclude that Calmette's serum is quite inactive against the venom of Echis carinata.

The outcome, then, of all these observations is to prove conclusively that, while the serum prepared by Calmette at Lille is of considerable value as a therapeutic measure in cases of cobra bite, if injected sufficiently early and in sufficient quantity, it is of no value whatever in the treatment of cases of bites from Daboia Russellii, Bungarus fasciatus, or Echis carinata.

We may now pass on to consider the experiments which were made to test the serum got from a horse immunised with the venom of the Australian tiger snake, against the venoms of some of the Indian snakes. This serum was, as I have mentioned above, prepared by Dr. Tidswell of Sydney, to whom I am greatly indebted for his kindness in sending me a considerable quantity. I have also stated above that Tidswell found this serum to be strongly antitoxic to the venom with which it was prepared, but to be quite inactive towards the venoms of three other Australian snakes. The results, therefore, of the observations which I have now to record, made with this serum and the poisons of three Indian snakes, are of the greatest theoretical interest.

The serum was first tested with the venom of the cobra. For these experiments rats were the animals employed. The test dose of venom used was ten lethal doses, *viz.*, 0·7 milligramme.[12] This test dose and different quantities of serum were mixed *in vitro*. The mixture was allowed to stand at laboratory temperature for half an hour. It was then injected subcutaneously into the inner side of the thigh. A reference to the protocols (series No. V) will show that the rat which received 4 c. c. of serum along with the test dose died after practically the same interval of time as one of the controls. The serum, therefore, had failed to neutralise any of the venom.

Tidswell's hoplocephalus serum was next tested with the venom of Bungarus fasciatus. For this series of experiments rabbits were the animals employed. I have already stated that the minimum lethal dose of this poison for a rabbit is 0·7 milligramme per kilo. when injected intravenously. The test dose of venom now used was 4 milligrammes per kilo., *viz.*, about 6 lethal doses. This test dose and different quantities of serum were mixed *in vitro*. The mixture was allowed to stand at laboratory temperature for half an hour. It was then injected into the marginal vein of the ear of a rabbit. It will be seen from the protocols (series No. VI) that the rabbit which received 5 c. c. of serum along with the test dose died in even a shorter time than the control which received no serum. We have, therefore, to conclude that the serum failed to neutralise even a modicum of the venom.

Lastly, the serum prepared by Dr. Tidswell was tested with the venom of

Protocols.

SERIES NO. I.—*Experiments to determine the minimum lethal dose for rabbits by intravenous injection of the pure venom of Bungarus fasciatus (banded krait).*

The poison was carefully dried fresh venom of Bungarus fasciatus. A 1 per cent. solution in sterile normal salt solution was made. Five-fold and ten-fold dilutions of this original solution were prepared when necessary. All the solutions were left unheated. The injections were made into the marginal vein of the ear.

The following were the results obtained :—

Animal.	Weight.	Amount of venom per kilo.	Result.
Rabbit 1	1,700 grammes	10 milligrammes	Died in 2¾ minutes.
„ 2	1,640 „	5 „	„ „ 18 „
„ 3	1,500 „	3·5 „	„ „ 3½ hours.
„ 4	1,600 „	3 „	„ „ 1 hour.
„ 5	1,370 „	2·5 „	Found dead after 20 hours.
„ 6	1,520 „	1 „	Died in 24 hours.
„ 7	1,470 „	1 „	„ „ 30 „
„ 8	1,590 „	0·8 „	„ „ 120 „
„ 9	1,760 „	0·75 „	Found dead after 66 hours.
„ 10	1,280 „	0·75 „	Died in 48 hours.
„ 11	1,680 „	0·7 „	Found dead after 48 hours.
„ 12	1,380 „	0·6 „	Ill : lost weight : recovered.
„ 13	1,830 „	0·5 „	Ill : lost weight : recovered.
„ 14	1,340 „	0·25 „	Slightly ill : recovered

From the above table it appears that about 0·7 milligramme per kilo. of the venom of this snake is the minimum lethal dose for a rabbit when injected intravenously.

SERIES NO. II.—*Experiments to ascertain if Calmette's serum has any neutralising power for the venom of Bungarus fasciatus.*

The serum used in these series of experiments was perfectly fresh serum. It had been received direct from Lille a few days before the observations were made. Two series of experiments were performed.

A. In the first series the test dose of venom used was 4 milligrammes per kilo., *viz.*, about 6 lethal doses. This test dose of venom in solution (1 per cent.) in normal salt solution and the serum in different quantities were mixed *in vitro*.

The mixture was allowed to stand at laboratory temperature (about 25° C.) for half an hour. It was then injected into the marginal vein of the ear of the rabbit.

The following were the results obtained :—

Animal.	Weight.	Amount of venom per kilo.	Amount of of serum.	Result.
Rabbit 1	1,180 grammes	4 milligrammes	0·5 c. c.	Died in 27 minutes.
„ 2	1,890 „	4 „	1 c. c.	„ „ 34 „
„ 3	1,570 „	4 „	2 c. c.	„ „ 2½ „
„ 4	1,810 „	4 „	5 c. c.	Found dead after 50 minutes.
„ 5 (Control.)	1,490 „	4 „	*Nil.*	Died in 30 minutes.

From this series of observations it will be seen that 5 c. c. of serum failed to neutralise 7·24—1·08 milligrammes = 6·16 milligrammes of venom.

B. In the second series of experiments with Calmette's serum and the venom of Bungarus fasciatus the test dose of venom employed was 2 milligrammes per kilo., *vis.*, about 3 lethal doses.

With this exception the same technique was used as in the first series of observations with this poison.

The following results were obtained :—

Animal.	Weight.	Amount of venom per kilo.	Amount of serum.	Result.
Rabbit 1	1,300 grammes	2 milligrammes	2 c. c.	Died in 18 hours.
„ 2	1,460 „	2 „	3 c. c.	Found dead after 17 hours.
„ 3	1,370 „	2 „	4 c. c.	„ „ „ 60 „
„ 4	1,200 „	2 „	5 c. c.	„ „ „ 40 „
„ 5 (Control.)	1,320 „	2 „	*Nil.*	„ „ „ 18 „

From this series of experiments it is evident that 5 c. c. of serum failed to neutralise 2·4—0·72 milligrammes = 1·68 milligrammes of this venom.

These two series of observations show positively that Calmette's serum is inactive against the venom of Bungarus fasciatus.

SERIES NO. III.—*Experiments to determine the minimum lethal dose for rabbits by intravenous injection of the venom of Echis carinata.*

The poison was carefully dried fresh venom of Echis carinata. A solution (0·1 per cent.) was made in normal salt solution.

When necessary, a ten-fold dilution of this solution was prepared. These solutions were left unheated. All injections were made into the marginal vein of the ear of the rabbit.

The following were the results obtained:—

Animal.	Weight.	Amount of venom per kilo.	Result.
Rabbit 1	1,360 grammes	1 milligramme	Died in 1½ minutes.
„ 2	1,520 „	0·5 „	„ „ 3½ „
„ 3	1,750 „	0·1 „	„ „ 6½ „
„ 4	1,540 „	0·05 „	„ „ 2 „
„ 5	1,580 „	0·05 „	„ „ 11½ „
„ 6	1,360 „	0·03 „	Lost weight: recovered.
„ 7	1,660 „ „	0·01 „	No symptoms.

From the above series of experiments it would appear that about 0·05 milligramme per kilo., injected intravenously into a rabbit, is the minimum lethal dose of this poison.

SERIES NO. IV.—*Experiments to ascertain if Calmette's serum has any neutralising power for the venom of Echis carinata.*

The serum employed was fresh serum recently imported from Lille. Rabbits were the animals used. The test dose of venom which was used was 0·05 milligramme per kilo., *viz.*, a single lethal dose.

Two experiments were performed.

A. In the first experiment this test dose and 4 c. c. of serum were mixed *in vitro*. The mixture was injected, soon after its preparation, into the marginal vein of the ear of a rabbit.

The following was the result:—

Animal.	Weight.	Amount of venom per kilo.	Amount of serum.	Result.
Rabbit	1,470 grammes	0·05 milligramme	4 c. c.	Died in 2 minutes.

B. In the second experiment 5 c. c. of serum were injected into the marginal vein of one ear. Six minutes afterwards the test dose of venom was injected into the marginal vein of the other ear.

The following was the result :—

Animal.	Weight.	Amount of venom per kilo.	Amount of serum.	Result.
Rabbit .	1,490 grammes .	0·05 milligramme .	5 c. c. .	Died in 2 minutes.

These two observations conclusively show that Calmette's anti-venomous serum is quite inactive against the venom of Echis carinata.

SERIES NO. V.—*Experiments to ascertain if the serum of a horse immunised with the pure venom of Hoplocephalus curtus has the power of neutralising cobra venom.*

The serum used was got, as explained in the text, from Dr. Tidswell of Sydney. Rats were the animals employed. Care was taken to select only those rats of about 115 grammes in weight. The test dose of cobra venom used in these observations was 0·7 milligramme, *viz.*, ten lethal doses of the sample of venom employed (*vide* note 12).

This test dose (0·1 per cent. solution) and the serum in different quantities were mixed *in vitro*.

The mixture was allowed to stand at laboratory temperature for half an hour. It was then injected subcutaneously in the inner side of the thigh.

The following was the result obtained :—

Animal.	Amount of venom.	Amount of serum.	Result.
Rat 1 .	0·7 milligramme . .	1 c. c.	Died in 1¼ hours.
„ 2	0·7 „ . .	3 c. c.	„ „ 51 minutes.
„ 3 .	0·7 „ . .	5 c. c.	„ „ 1½ hours.
„ 4 (Control.)	0·7 „ . .	*Nil.*	„ „ 1½ „
„ 5 . (Control.)	0·7 „ . .	*Nil.*	„ „ 52 minutes.

From this series of experiments it is evident that Tidswell's hoplocephalus serum has no power to neutralise cobra venom.

SERIES NO. VI.—*Experiments to ascertain if the serum of a horse immunised with the venom of Hoplocephalus curtus has the power to neutralise the venom of Bungarus fasciatus.*

The same serum as was used in the experiments tabulated in Series No. V was employed. The observations were made on rabbits. The test dose of venom was the same as was used in the experiments of Series No. II A, *viz.*, 4 milligrammes per kilo., that is to say, about 6 lethal doses. In each instance this test dose (1 per cent. solution) and the serum in different quantities were mixed *in vitro.*

The mixture was allowed to stand at laboratory temperature for half an hour. In was then injected into the marginal vein of the ear of the rabbit.

The following were the results obtained :—

Animal.	Weight.	Amount of venom per kilo.	Amount of serum.	Result.
Rabbit 1	1,450 grammes	4 milligrammes	0·5 c. c.	Died in 32 minutes.
„ 2	1,780 „	4 „	1 c. c.	„ „ 12 „
„ 3	1,460 „	4 „	2 c. c.	„ „ 22 „
„ 4	1,680 „	4 „	5 c. c.	„ „ 10 „
„ 5 (Control.)	1,490 „	4 „	*Nil.*	„ „ 30 „

From this series of experiments it is at once evident that the serum of a horse immunised with the venom of Hoplocephalus curtus has no power to neutralise the venom of Bungarus fasciatus.

SERIES NO. VII.—*Experiments to ascertain if the serum of a horse immunised with the venom of Hoplocephalus curtus has the power to neutralise the venom of Daboia Russellii.*

The serum which was used for these experiments was the same as was employed for the experiments tabulated in Series Nos. V and VI. Rabbits were the animals used.

Two series of experiments were performed.

A. In the first series of observations the test dose of venom employed was 0·5 milligramme per kilo., *viz.*, an amount which, as regards this sample of venom, did not cause intravascular thrombosis when injected intravenously. This test dose and varying quantities of serum were mixed *in vitro.* The mixture was allowed to stand at laboratory temperature for half an hour.

It was then injected into the marginal vein of the ear of the rabbit.

The following were the results obtained :—

Animal.	Weight.	Amount of venom per kilo.	Amount of serum.	Result.
Rabbit 1	1,480 grammes	0·5 milligramme	1 c. c.	Found dead after 18 hours
„ 2	1,680 „	0·5 „	2 c. c.	Ditto.
„ 3	1,400 „	0·5 „	3 c. c.	Ditto.
„ 4	1,510 „	0·5 „	4 c. c.	Ditto.
„ 5 (Control.)	1,280 „	0·5 „	*Nil.*	Ditto.

B. In the second series of experiments a different sample of venom was used. Of this sample it was found that the minimum amount which caused intravascular thrombosis, when injected intravenously into a rabbit, was 0·2 milligramme per kilo. A single experiment was made with this sample. The test dose used was 0·6 milligramme per kilo., *viz.*, three times the amount which could cause intravascular thrombosis.

The technique was as follows:—5 c. c. of serum were injected into the marginal vein of one ear: five minutes afterwards the test dose of venom (0·1% solution) was injected into the marginal vein of the other ear.

The following was the result obtained:—

Animal.	Weight.	Amount of venom per kilo.	Amount of serum.	Result.
Rabbit	1,380 grammes	0·6 milligramme	5 c. c.	Died in 1½ minutes.

From these series of observations it is therefore evident that the serum of a horse immunised with the venom of Hoplocephalus curtus has no power to neutralise the venom of Daboia Russellii.

Notes and references.

1. The information used in this paper concerning the classification of the poisonous snakes was taken from 'Fauna of British India—Reptilia and Batrachia' by Boulenger.

2. Calmette: 'Le venin des Serpents': Paris, 1896. 'Notice sur le Sérum Antivenimeux et sur le Traitement des Morsures de Serpents': Lille, 1901.

3. 'Journal of Pathology and Bacteriology': Edinburgh and London: 1902, Vol. VIII, page 1.

Paper: 'On the action of the venoms of the cobra (Naia tripudians) and of the daboia (Daboia Russellii) on the red blood corpuscles and on the blood plasma': in process of publication by the Government of India in Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India, New Series.

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10. *Loc. Cit.*

11. 'Journal of Pathology and Bacteriology': Edinburgh and London: 1902, Vol. VIII, page 1.

12. The minimum lethal dose for rats of about 115 grammes weight of the sample of venom used for these experiments was found to be 0·07 milligramme. In a previous paper (*vide* reference 6), I stated that the minimum lethal dose for rats of this weight was 0·04 milligramme. A different sample of venom, however, had been used for these first observations. It is evident, therefore, that, as different samples of venom vary in toxicity, for each sample the minimum lethal dose should be determined for all experiments having in view the testing of an antitoxic serum.

13. 'Lancet': August 16th, 1902.